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RESEARCH

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Isolation and identification of antagonistic fungi on coffee leaf rust in the Dieng highlands of Banjarnegara, Indonesia

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Abstract

Background Coffee is an important plantation crop in Indonesia. The coffee cultivation process was disrupted due to the fungus *Hemileia vastatrix* which causes rust disease. Biological control has the potential to suppress disease development. The diversity of antagonistic fungi such as *Trichoderma* in nature is very abundant, so it is necessary to explore and find *Trichoderma* which has the potential as a biological agent in controlling coffee leaf rust. The research aimed to obtain antagonist fungi that have the potential as biological agents in controlling coffee leaf rust naturally in the coffee plant ecosystem.

Result Morphology of a local antagonist fungus isolate, coded TBK1, was identified as *Trichoderma atroviride* which had the potential to naturally control coffee leaf rust by *H. vastatrix* through a mycoparasitic mechanism.

Conclusion In the Dieng Plateau, Banjarnegara Indonesia, an antagonistic fungus as a biological agent, *T. atroviride*, was found to control *H. vastatrix* coffee leaf rust.

Keywords Antagonistic fungi, *Hemileia vastatrix*, Leaf rust coffee, *Trichoderma atroviride*

Background

Arabica coffee is widely cultivated in Indonesia and is an important commodity in supporting the Indonesian economy (Fithriyyah et al. 2020). *Hemileia vastatrix* is the cause of coffee leaf rust disease. The disease can develop from nurseries to production plants. This will cause a decrease and loss of yield and interfere with plant metabolism in providing photosynthates through the destruction of leaves. Disturbance of plants due to rust disease is indicated by the presence of light yellow to dark yellow spots on the abaxial leaves, and on the adaxial it is indicated by a powdery mass consisting of rust fungus urediospores. The stain gradually enlarges and produces uredospore. Apical symptoms appear as chlorosis spots, yellowish without sporulation. Leaf rust in advanced infection causes the leaves to fall and the plants to be bare.

Coffee leaf rust disease by *H. vastatrix* was first reported in Sri Lanka in 1869. Then it spread to Indonesia (Java, Sumatra), Philippines, Angola, Brazil, Nicaragua, Panama, El Salvador, Guatemala, and Mexico (Filho and Domian 2019). The most vulnerable coffee species is Arabica coffee. Yield losses due to coffee leaf rust disease reached 25%, and disease severity was up to 45% in Mandailing Natal District, North Sumatra (Siska et al. 2018). *H. vastatrix* is a biotrophic organism known as an obligate parasite that eats living cells or can only live in living cells and cannot be cultured in artificial media (Porto et al. 2019). Uredospores are reproductive structures of *H. vastatrix* (asexual reproduction) which function as a means of reproduction and infect coffee leaves. The structure of the uredospores is kidney-shaped, rough

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like serrated on top, while smooth in the middle. Spore germination requires adequate environmental conditions, such as constant rainfall, temperatures 18–24 °C, and a dark environment (Morales-Antonio et al. 2021). *H. vastatrix* lasted 5.3–8.5 h to form an appressorium. Germination was inhibited by light and dry conditions (Hocking 1968).

Biological control of coffee leaf rust needs to be done with alternatives that support safety and are environmentally friendly. Biological control has the potential to reduce damage caused by pathogens and is not harmful to the environment (Raza et al. 2021). One of the biological control agents that can be used is *Trichoderma* (Mukhopadhyay and Kumar 2020). *Trichoderma* is abundant in nature; there are more than 100 species (Poveda 2021). *Trichoderma* has mycoparasite, competition, and antibiosis mechanisms (Sood et al. 2020), and produces enzymes and antibiotics. *Trichoderma* can stimulate plant development and growth, as well as induce plant systemic resistance in suppressing pathogenic activity (Kumar et al. 2017). The fungal pathogens, namely *Colletotrichum capsici* and *C. gloeosporioides*, can be controlled by *Bacillus subtilis* B298, with an antibiosis mechanism, and can increase plant growth (Prihatiningsih et al. 2019a). *B. subtilis* B298 microcapsule formula can reduce disease intensity by 48% with an infection rate of 0.02 unit.day-1. Microencapsulation of *B. subtilis* B298 had a mechanism of inducing plant systemic disease resistance in chili by increasing total plant phenol levels (Prihatiningsih et al. 2019b).

The purpose of this study was to obtain antagonistic fungi as a biological agent in controlling coffee leaf rust naturally.

Methods

Isolation antagonists of *Hemileia vastatrix*

A survey for the antagonist of the fungus *H. vastatrix* was conducted in Indonesia, focusing on the Dieng Mountains, Banjarnegara district, representing the geographically indicated coffee plant center areas of the Dieng Plateau Area with Arabica coffee varieties. Coffee is cultivated naturally and conventionally with chemical pesticide control. On rust spots by *H. vastatrix*, antagonistic fungi were also found on the surface of the spots which grew simultaneously on the coffee philosphere. Antagonistic fungal cultures were obtained through direct isolation of white mycelium from antagonistic fungi attached to the surface of rust spots by *H. vastatrix* (Kapeua-Ndacnou et al. 2023). The mycelium was taken with sterile adhesive, from colonized leaf rust spores and affixed to a glass object and then observed for its morphology with a microscope. After 10 days of growth on potato dextrose agar (PDA) medium, the antagonistic colony characters were recorded. Colony morphologies were compared based on standard terminology (Lodge et al. 2004). After 10 days of incubation in Petri dishes, the colony diameter was measured.

Isolation of antagonistic fungi was carried out by taking the spores and then transferring them to PDA medium which had previously been added with streptomycin to suppress bacterial growth and other contamination. Colonies appeared after 3 days, white in color, and grew light green spores, and filled the Petri dish containing PDA after 10 days of culture. The culture was purified by taking the target colony and growing back on the PDA medium. To identify *H. vastatrix* uredinia colonization, sections of coffee leaf samples were obtained at the beginning of the exploratory study and appeared to be colonized by antagonist fungi as indicated by the presence of white color on the leaf rust.

Identification

DNA Extraction and Sequences—Phylogenetic Analyses TBK1 isolates that colonize rust were identified molecularly based on partial genetic analysis of the internal locus of transcribed spacer (ITS) fungal ribosomal DNA. The TBK1 isolate to be identified was grown on PDA media. Fungal DNA extraction was carried out with the Quick-DNA Fungal/Bacterial Miniprep Kit (Zymo Research 2021). This method is also used by (Minarni et al. 2021).

Raw data from sequencing results were then sorted and assembled, using the BioEdit application (http://www.mbio.ncsu.edu/BioEdit/bioedit.html). Next was the sequence result included in the BLAST registered with the National Center for Biotechnology information on the website (http://www.ncbi.nlm.nih.gov/BLAST). This aim was to determine the genus or species that have similarities/homology and molecularly.

Sequences—Phylogenetic analyses are carried out to measure DNA concentrations using the Gene spec tool. DNA duplication, also known as sequencing The TaKaRa PCR Thermal Cycler tool was used to complete the cycle procedure utilizing the bi-directional squeezing technique. Setting was carried out at 96 °C for 10 s, then annealing at 50 °C for 5 s, and extension at 60 °C for 4 min, and repetitions were carried out 25 times.

Ethanol precipitation can also be called product sequence purification. The DNA sample was flashed and transferred to a new microtube, then added 64 µl Ethanol 99.5% (room temperature), then vortexed and added 16 µl H2O, then vortex again. The sample was wrapped in aluminum for 10 min. Samples were centrifuged at 28 °C for 15 min at 14,000 atm. All upper layers were discarded, and 100 µl ethanol 70% (room temperature) was added. The sample was centrifuged again at 28 °C for

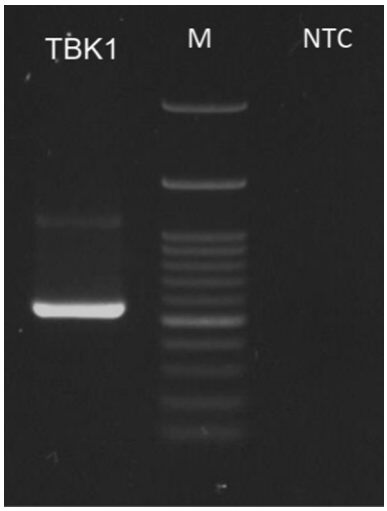

Fig. 1 DNA banding pattern of *Trichoderma* TBK1 isolate as a result of electrophoresis, **TBK1**. Sample isolate of *Trichoderma* TBK1 **M**. DNA ladder, **NTC**. Nontemplate control

10 min at 14,000 atm. All upper layers were removed and dried wrapped in aluminum foil for 15 min (room temperature). Samples were analyzed at Genetics Science, Serpong, Tangerang Banten. Mushroom isolates that had been subcultured were identified to the level of genus taxa that use the BLAST bioinformatics method and are available online on the website https://blast.ncbi.nlm.nih.gov/Blast.cgi.

Biocontrol mechanism isolate TBK1 (Kalibening)

The main mechanism of biocontrol TBK1 isolate (Kalibening) was mycoparasitism. These data were obtained by observing the spores of isolate TBK1 (Kalibening) which colonized *H. vastatrix* uredinia. Observations were made under a light microscope.

Results

Isolation antagonists of *Hemileia vastatrix*

Coffee leaf rust can reduce production and cause endemic under favorable environmental conditions. An environment with an average humidity of 40% and shady branches without pruning treatment is very favorable for rust disease so that it accelerates development. Leaf moisture can maintain optimum temperature and higher relative humidity which can support the germination of pathogenic spores. Under conditions of full sun exposure, it can reduce spore growth. Shade supports the development of antagonistic fungi as natural enemies of *H. vastatrix* coffee leaf rust.

Morphological and molecular identification

Morphological identification Antagonistic fungi isolated TBK1 (Kalibening) from coffee leaf rust colonized by antagonistic fungi. Fungus purification was done by growing it on PDA media. From the observation of morphological characteristics, the isolates found were identified as *T. atroviride* (Fig. 1). Complete morphological characters are listed in (Table 1 and Fig. 2).

Molecular identification PCR analysis using universal primers, namely ITS1-ITS4 primers, showed that the TBK1 antagonist DNA had a DNA band weighing 594 bp (Fig. 3), by the results of the study (Naef et al. 2005).

BLASTN search was applied to sequences from different presumed regions, which revealed that TBK1 isolate belongs to the genus *Trichoderma,* which had 100% base pair similarity to ITS 1, 5.8S ribosomal RNA sequence belonging to *T. atroviride* strain 10,770. This isolate was isolated as an antagonist from Arabica coffee leaves. The isolate grew on *H. vastatrix* uredinia rust (Table 2).

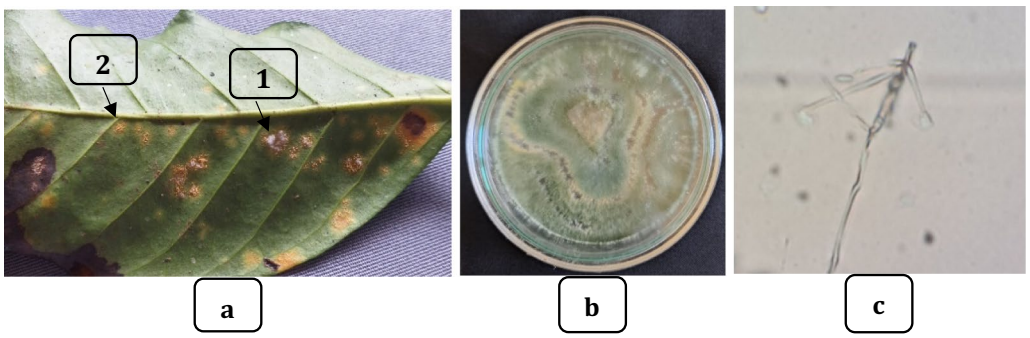

Fig. 2 In *planta* and in *vitro* effects of isolate TBK1 (Kalibening) antagonist/biocontrol on *Hemileia vastatrix*, **a** (1) leaf rust coffee isolate TBK1 was inoculated on **a** (2) *H. vastatrix*, **b** coloni of isolate TBK1 on PDA after 5 days, **c** microscopic observation of the isolate TBK1 showing phialidesand conidia (Yan et al. 2019)

Table 1 Morphological characteristics of antagonistic fungi TBK1 (Kalibening) Isolate

Isolate	Color and shape of the colony	Conidial form	Conidial color	Genus	References
TBK1	White, conidia were more or less restricted to concentric rings. Shape codia ellipsoidal	Oval, attached to shoots and body conidiophores (branches), after the hyphae cross, conidial growth in groups	Hyaline	*Trichoderma* (Fig. 1)	Yan et al. (2019); Kim et al. (2016)

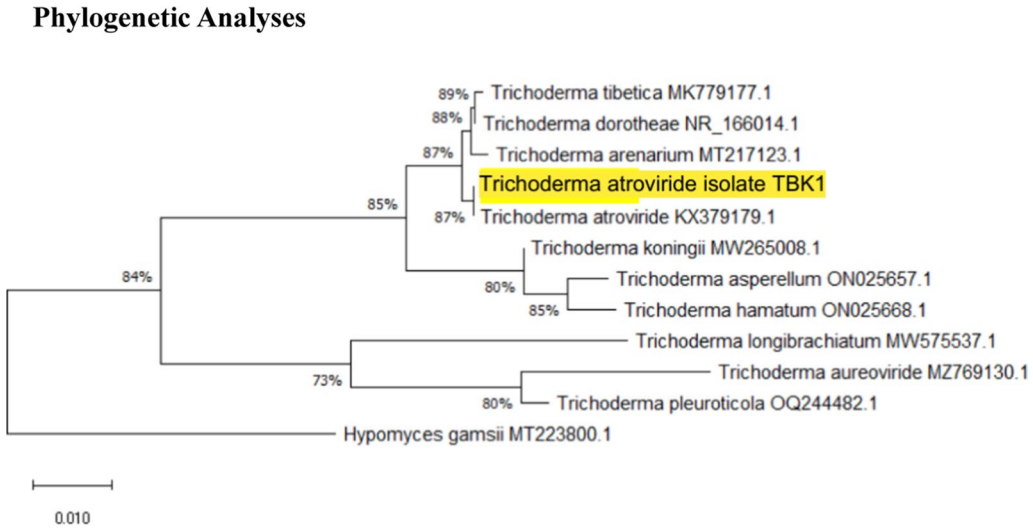

Fig. 3 Phylogenetic tree of *Trichoderma atroviride* isolate TBK1 based on ITS sequence. New isolates are marked in yellow. The number on the branch indicates 84% bootstrap compatibility, and the scale bar indicates nucleotide substitution per site

Table 2 Results of a fungal taxon that generates BLAST ITS1 DNA homology in NCBI (https://www.ncbi.nlm.nih.gov/)

Isolate	Type	No accession/host	DNA size (bp)	Query coverage (%)	E value	Per. Ident (%)	Total score
TBK1/host/size Hemileia vastatrix/594 bp	*Trichoderma atriviride* strain 10,770	KX379179.1	599BP	100%	0	100	1098

Phylogenetic analyses

Mechanism antagonist of Trichoderma atroviride isolate TBK1

Pathogens can be controlled by safe methods, namely using microorganisms. The results of this study indicated that there were antagonistic mechanisms such as mycoparasitism against *H. vastatrix* which can operate independently or together to suppress plant pathogens (Fig. 4).

Discussion

Biocontrol agents were found to be reducing the intensity of Arabica coffee leaf rust under field conditions. The biocontrol agent TBK1 (Kalibening) was identified as *T. atroviride* based on molecular identification. *T. atroviride* was reported as a cosmopolitan species isolated from soil and rhizosphere (Dodd et al. 2017). Rodríguez et al. (2021) reported for the first time in Kenya, Western Region, Nandi District, Kakamega Protected Forest, Isecheno, a coffee plantation area with an altitude of 1600 m above sea level and isolated as an endophyte from Coffea arabica leaves.

In this research, *T. atroviride* isolate TBK1 was found in the tropical climate of Indonesia, the Dieng Plateau Region, Banjarnegara Regency, Indonesia, at an altitude of 1,049 m above sea level, an average humidity of 80% and a wind speed of 20 km/hour (https://banjarnegarakab.bps.go.id/). Colonies of *T. atroviride* isolate TBK1 were isolated from the phyllosphere of coffee leaves which were colonized by *H. vastatrix*, and appeared white above the uredospores colonies of *H. vastatrix* which were orange in color on coffee leaves. *T. atroviride* strain 10,770 was previously discovered by Van der Linde,

E.J., in 2016 on the host *Agaricus bisporus* and uploaded to the NCBI gene bank (https://www.ncbi.nlm.nih.gov/nuccore/KX379179.1).

Trichoderma atroviride can also parasitize pathogens in fruit and endophytic tissues (Rodríguez et al. 2021). In addition to functioning as a biocontrol agent for *H. vastatrix, T. atroviride* was reported to suppress the growth of the pathogen *Fusarium graminearum* (Naef et al. 2005), a biocontrol agent for the green mold of *Ganoderma* (Yan et al. 2019), and a biocontrol agent for *Rhizoctonia* and *Pythium* (Brunner et al. 2005).

Trichoderma atroviride TBK1 isolate was grown in a PDA medium and incubated at 30 °C. The results of observing the morphological characteristics of TBK1 isolates showed color and shape colony, conidiophore shape, and conidian shape (Fig. 2b and c; Table 1). The results of observations of *T. atroviride* isolate TBK1 colonies formed concentric spores, dark green in color in each circle. Isolated colonies grew uniformly on the surface of the medium and conidia formed at the ends of the Petri dishes. It is characterized by a slightly yellowish dark green color and white mycelium (Fig. 2b). Conidiophores were unilateral and paired. The shape of the phialids was straight or winding, cylindrical, and at the ends of the phialids narrow, the conidia are oval (Kalimutu et al. 2020).

DNA bands measuring 594 bp were successfully amplified from TBK1 and ITS4 isolates. This is in line with the research results of *(*Hermosa et al. 2000*).* Another study obtained amplified DNA fragment sizes of 600 bp (Matas-Baca et al. 2022). The band sequence was then used for DNA sequencing analysis. The results of DNA sequencing revealed that the TBK1 isolate sequence had 100% base pair similarity with the ITS 1, 5.8S ribosomal RNA sequence belonging to *T. atroviride* strain 10,770. From the results of the analysis above, it could be concluded with a high percentage of similarity with a bit score above 700 and an e-value of 0. The bit score was a measure used for juxtaposition. E-value was an estimated value that provides a statistically significant measure of the two sequences. The highest e-value indicated the lowest homology level, and for the lowest e-value 0 (zero), the sequence was identical. The two isolates similarity is indicated by their greatest bit score (Claverie and Notredame 2007).

The results of ITS DNA sequence analysis based on the database in the BLASTN program showed that TBK1 isolate had the closest kinship with *T. dorotheae* NR_166014.1 with a bootstrap value of 88%; then, TBK1 isolate had identical similarities with *T. atroviride* KX379179.1 and *T. arenarium* MT217123.1 with a bootstrap value of 87%. The phylogenetic analysis from this research supports the data regarding the morphological characteristics that allow the identification of *T. atroviride* isolates. Using ITS sequences and phylogenetic analysis can be used to identify new species of *Trichoderma* that exists in agricultural agroecosystems (Asis et al. 2021) and coffee plant agroecosystem (Khairillah et al. 2021).

Biological disease control has been widely developed. Biological control is a plant symbiont with a pathogenic community that maintains plants and controls pathogen populations in various environmental conditions. *Trichoderma* spp. have been widely used as biocontrol agents for biological control of plant pathogens. The main strategy in biological control is that *Trichoderma* can fight pathogens with mycoparasitism (Karuppiah et al. 2019).

Mycoparasitism is one of the main mechanisms of inhibiting the growth of mycelium of fungal pathogens, providing nutrition for mycoparasites when they attack

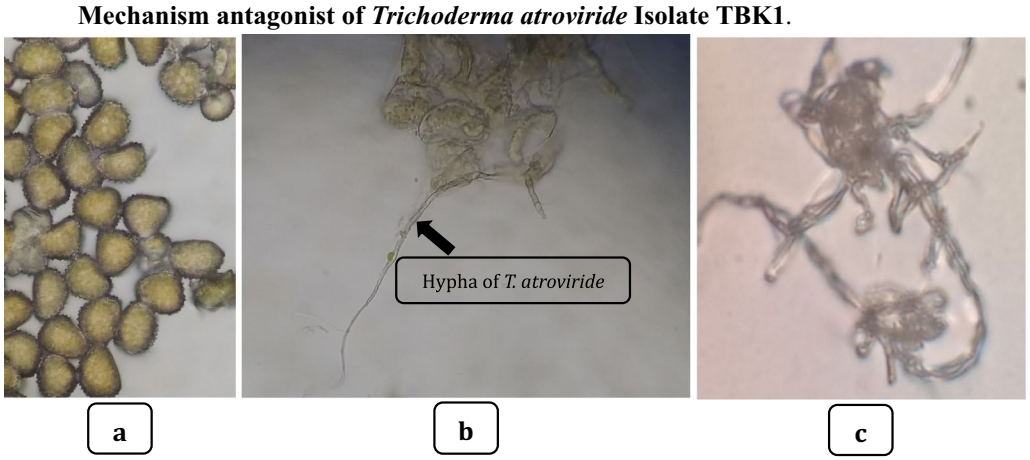

Fig. 4 Mycoparasitism by *Trichoderma atroviride* isolate TBK1; **a** pure *Hemileia vastatrix* uredospore; **bc** *T. atroviride* TBK1 parasitizes *Hemileia vastatrix*

pathogens. This mechanism can cause cell wall damage in the *H. vastatrix* uredospore. This is because *T. atroviride* isolates TBK1 and binds to the pathogen *H. vastatrix* through the production of cell wall damaging enzymes such as 1,3-glucanase, chitinase, protease, and cellulase (Guzm et al. 2023). Secondary metabolite 6-pentyl-2-pyrone has *T. atroviride* strain AT10 (Alvarez et al. 2022). Before physical contact in the mycoparasite interaction between *Trichoderma* and fungal pathogens, diffusion factors are released from the host to induce hydrolytic enzymes. During direct contact, host cell wall lectins can induce *Trichoderma* around the host, thereby encouraging the formation of hyphae and appresorium to destroy pathogens (Zeilinger and Omann 2007).

Trichoderma atroviride isolates TBK1 showed mycoparasitic activity in the presence of mycelial invasion of *H. vastatrix* when isolated from pure coffee leaf cultures. Microscopic observations made at the contact area between *T. atroviride* isolate TBK1 and *H. vastatrix* showed a change in the shape of the cell wall of the *H. vastatrix* uredospore characterized by lysis of the cell wall which appeared thin and transparent different from pure uredospores. At an advanced stage, the mycelium of *T. atroviride* isolate TBK1 covered all parts of the uredospores causing damage and unable to germinate. The interaction of *Trichoderma* with the pathogens *Rhizoctonia* and *Phytium* showed that *Trichoderma* grows parallel to the pathogen and develops around the pathogen. Degradation of the pathogen will occur after *Trichoderma* penetrates with appressorium (Benhamou and Chet 1993). Application of *Trichoderma* can reduce the percentage of disease occurrence, the severity of disease caused by the *H. vastatrix* pathogen (Mamani-Huayhua et al. 2021).

Conclusion

The results of molecular identification of the antagonistic fungus from coffee leaf rust as *Trichoderma atroviride*. The control mechanism is mycoparasite which can grow on *H. vastatrix* urediospores, causing malformations in the form of changes in the cell wall and inhibiting urediospores germination. The obtained *T. atroviride* had prospects for development as a biological agent against coffee leaf rust.

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Author contributions
WNK conceived and designed the study, drafted the manuscript, and analyzed and interpreted the data; WNK, UDR, WW, and RA acquired the data; PN revised the manuscript critically for important intellectual content. All authors read and approved the final manuscript.

Availability of data and materials
All data are available in the article and the materials used in this work are of high quality and grade. The sequencing data generated and analyzed in this study are available at NCBI database Sequence Read Archive (https://www.ncbi.nlm.nih.gov/nuccore/) Accession Number: KX379179.1, OL631238.1, KC884783.1, AB63320, 4.1, MK871201.1, MK871197.1, MK870815.1, MK870814.1, MK870175.1, MH153623.1.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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References

Alvarez F, Arena M, Auteri D et al (2022) Peer review of the pesticide risk assessment of the active substance *Trichoderma atroviride* strain AT10. EFSA J 20:1–19. https://doi.org/10.2903/j.efsa.2022.7200

Asis A, Ahmed S, Laila S et al (2021) Identification patterns of *Trichoderma* strains using morphological characteristics, phylogenetic analyses and lignocellulolytic activities. Mol Biol Rep 48:3285–3301. https://doi.org/10.1007/s11033-021-06321-0

Benhamou N, Chet I (1993) Hyphal interactions between *Trichoderma harzianum* and *Rhizoctonia solani*: ultrastructure and gold cytochemistry of the mycoparasitic process. Phytopathology 83:1062–1071

Brunner K, Zeilinger S, Ciliento R et al (2005) Improvement of the fungal biocontrol agent *Trichoderma atroviride* to enhance both antagonism and induction of plant systemic disease resistance. Appl Environ Microbiol 71:3959–3965. https://doi.org/10.1128/AEM.71.7.3959-3965.2005

Claverie J-M, Notredame C (2007) Bioinformatics for dummies. Wiley Publishing, Inc

Dodd SL, Lieckfeldt E, Samuels GJ et al (2017) *Hypocrea atroviridis* sp. nov., the teleomorph of *Trichoderma atroviride*. Mycologia 5514:26–40. https://doi.org/10.1080/15572536.2004.11833129

Filho E de V, Domian CA (2019) Prevention and Control of Coffee Leaf Rust. Tropical Agricultural Research and Higher Education Center (CATIE), Costa Rika

Fithriyyah D, Wulandari E, Sendjaja TP (2020) potensi komoditas kopi dalam perekonomian daerah di Kecamatan Pangalengan Kabupaten Bandung. Mimb Agribisnis J Pemikir Masy Ilm Berwawasan Agribisnis 6:700. https://doi.org/10.25157/ma.v6i2.3408

Guzm P, Kumar A, Santos-villalobos SDL et al (2023) *Trichoderma* species: our best fungal allies in the biocontrol of plant diseases—a review. Planta 12:1–35. https://doi.org/10.3390/plants12030432

Hermosa MR, Grondona I, Iturriaga EA (2000) Molecular Characterization and Identification of Biocontrol Isolates of *Trichoderma* spp. Appl Environ Microbiol 66:1890–1898

Hocking D (1968) Effects of light on germination and infection of coffee rust (*Hemileia vastatrix*). Trans Br Mycol Soc 51:89–93. https://doi.org/10.1016/s0007-1536(68)80124-x

Kalimutu PK, Komang Mahardika IB, Anak Agung Sagung PRA (2020) Antagonism test of *Trichoderma atroviride* and *Gliocladium* sp bali local isolates as a disease control of blendok disease (*Botryodiplodia theobromae*) in Grapefruit (*Citrus grandis* L. *Osbeck*). SEAS Sustain Environ Agric Sci 4:102–110

Kapeua-Ndacnou M, de Abreu LM, de Macedo DM et al (2023) Assessing the biocontrol potential of clonostachys species isolated as endophytes from coffea species and as mycoparasites of hemileia rusts of coffee in Africa. J Fungi. https://doi.org/10.3390/jof9020248

Karuppiah V, Sun J, Li T, Vallikkannu M (2019) Co-cultivation of *Trichoderma asperellum* GDFS1009 and *Bacillus amyloliquefaciens* 1841 causes differential gene expression and improvement in the wheat growth and biocontrol activity. Microbiology 10:1–16. https://doi.org/10.3389/fmicb.2019.01068

Khairillah YN, Sukarno N, Batubara I (2021) *Trichoderma hamatum* derived from coffee plant (*Coffea canephora*) rhizosphere inhibit *Candida albicans* Growth. J Biol Biol Educ 13:369–378. https://doi.org/10.15294/biosaintifika.v13i3.31132

Kim JY, Kwon HW, Yun YH, Kim SH (2016) Identification and characterization of *Trichoderma* species damaging shiitake mushroom bed-logs infested by camptomyia pest. J Microbiol Biotechnol 26:909–917. https://doi.org/10.4014/jmb.1602.02012

Kumar V, Kumar M, Sharma S, Prasad R (2017) Role of *Trichoderma* spp. as a biocontrol agent of fungal plant pathogens. Probiotics Plant Heal. https://doi.org/10.1007/978-981-10-3473-2

Lodge DJ, Ammirati JF, O'dell TE et al (2004) Biodiversity of Fungi. Elsevier Academic Press

Mamani-Huayhua G, Leon-Ttacca B, Palao-Iturregui LA, Borja-Loza YR (2021) Biocontrol of coffee yellow rust (*Hemileia vastatrix* Berk. & Br.) with *Trichoderma* sp. endophyte strains. Cultiv Trop 42

Matas-Baca MÁ, García CU, Pérez-Álvarez S et al (2022) Morphological and molecular characterization of a new autochthonous *Trichoderma* sp. isolate and its biocontrol efficacy against *Alternaria* sp. Saudi J Biol Sci 29:2620–2625. https://doi.org/10.1016/j.sjbs.2021.12.052

Minarni EW, Soesanto L, Suyanto A, Rostaman, (2021) Molecular identification of three entomopathogenic fungi infecting the brown plant hopper pest in Indonesia. Egypt J Biol Pest Control. https://doi.org/10.1186/s41938-021-00412-7

Morales-Antonio MA, Santiago-Martínez GM, Vásquez-López A et al (2021) Uredospore germination of *Hemileia vastatrix* and its inhibition by the effect of plant extracts in vitro. Int J Agric Nat Resour 48:108–114. https://doi.org/10.7764/ijanr.v48i2.2272

Mukhopadhyay R, Kumar D (2020) *Trichoderma*: a beneficial antifungal agent and insights into its mechanism of biocontrol potential. Egypt J Biol Pest Control. https://doi.org/10.1186/s41938-020-00333-x

Naef A, Senatore M, Défago G (2005) A microsatellite based method for quantification of fungi in decomposing plant material elucidates the role of *Fusarium graminearum* DON production in the saprophytic competition with *Trichoderma atroviride* in maize tissue microcosms. FEMS Microbiol Ecol 55:211–220. https://doi.org/10.1111/j.1574-6941.2005.00023.x

Porto BN, Caixeta ET, Mathioni SM et al (2019) Genome sequencing and transcript analysis of *Hemileia vastatrix* reveal expression dynamics of candidate effectors dependent on host compatibility. PLoS ONE 14:1–23. https://doi.org/10.1371/journal.pone.0215598

Poveda J (2021) *Trichoderma* as biocontrol agent against pests: new uses for a mycoparasite. Biol Control 159:104634. https://doi.org/10.1016/j.biocontrol.2021.104634

Prihatiningsih N, Djatmiko HA, Erminawati (2019a) Bio-management of anthracnose disease in chilli with microencapsulates containing *Bacillus subtilis* B298. In: IOP conference series: earth and environmental science, p 6

Prihatiningsih N, Djatmiko HA, Erminawati E, Lestari P (2019b) *Bacillus subtilis* from potato rhizosphere as biological control agent and chili growth promoter. J Perlindungan Tanam Indones 23:179. https://doi.org/10.22146/jpti.40606

Raza T, Yahya M, Mahmood S et al (2021) Biological management of selected weeds of wheat through co-application of allelopathic rhizobacteria and sorghum extract. Biol Control 164:104775. https://doi.org/10.1016/j.biocontrol.2021.104775

Rodríguez M, del Carmen H, Evans HC, de Abreu LM et al (2021) New species and records of *Trichoderma* isolated as mycoparasites and endophytes from cultivated and wild coffee in Africa. Sci Rep 11:1–30. https://doi.org/10.1038/s41598-021-84111-1

Siska RKW, Lubis L, Lisnawati L (2018) Serangan Karat Daun Kopi (*Hemileia vastatrix* B et Br) pada tanaman kopi arabika di perkebunan rakyat Kabupaten Mandailing Natal Sumatera Utara. In: Talenta conference series: agricultural and natural resources (ANR), pp 82–86

Sood M, Kapoor D, Kumar V, Sheteiwy MS (2020) *Trichoderma*: the "secrets" of a multitalented. Plants 9:762

Yan Y, Moodley O, Xu J (2019) Green mold caused by *Trichoderma atroviride* on the lingzhi medicinal mushroom, *Ganoderma lingzhi* (Agaricomycetes). Int J Med Mushrooms 21:515–521. https://doi.org/10.1615/IntJMedMushrooms.2019030352

Zeilinger S, Omann M (2007) *Trichoderma* biocontrol: signal transduction pathways involved in host sensing and mycoparasitism. Gene Regul Biol 1:227–234

Zymo Research (2021) Quick-DNA ™ Fungal/Bacterial Miniprep Kit. DNA from tough-to-lyse fungi and bacteria samples, pp 1–11

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